5A,17A/2009G136,2011G585

Structure of unliganded CRM1 reveals the mechanism of autoinhibition

Natsumi Saito[1] and Yoshiyuki Matsuura[*1,2]
[1]Division of Biological Science and [2]Structural Biology Research Center, Graduate School of Science, Nagoya Univeristy, Furo-cho, Chikusa-ku, Nagoya 464-8602, Japan

1 Introduction

Macromolecular exchange between the cytoplasm and the nucleus is an essential cellular process in eukaryotes. Nuclear transport occurs through nuclear pore complexes and is mediated in most cases by soluble transport receptors that belong to the karyopherin-β superfamily. CRM1 (also known as exportin 1 or Xpo1) is the most versatile nuclear export receptor that carries a broad range of cargoes from the nucleus to the cytoplasm. The majority of cargoes of CRM1 contain the so-called leucine-rich nuclear export signal (NES) that typically harbor four or five characteristically spaced hydrophobic residues. The small GTPase Ran and various Ran-binding proteins regulate loading and unloading of cargoes, and thereby determine the transport directionality. In the nucleus, RanGTP facilitates cargo association with CRM1, forming a ternary nuclear export complex that can translocates across the nuclear pore. In the cytoplasm, RanBP1/2 and RanGAP cooperate to dissociate the cargo and promote GTP hydrolysis on Ran.

Although previous crystallographic studies have defined the interactions between CRM1, NES, and RanGTP [1,2], the mechanism underlying the positive cooperativity in RanGTP and NES binding to CRM1 remained incompletely understood, due to lack of high resolution structure of unliganded CRM1. Herein we report a 2.1-Å-resolution crystal structure of unliganded *Saccharomyces cerevisiae* CRM1 (Xpo1p) that provides definitive structural data to establish the precise mechanism of autoinhibition of CRM1 [3].

2 Experiment

Crystals of unliganded CRM1 were grown by hanging drop vapor diffusion method. Preliminary X-ray diffraction experiments were carried out at Photon Factory and the datasets used for final structure determination were collected at SPring-8.

3 Results and Discussion

The structure of unliganded CRM1 was solved by selenomethionine (SeMet) multi-wavelength anomalous diffraction (MAD) phasing method and was refined to *R*-free of 21.7% (R_{cryst} 18.7%).

The HEAT repeats of unliganded CRM1 are organized into a ring-shaped molecule with the C-terminal helix (C-helix) lying across the ring (Fig. 1). The NES-binding cleft, formed between the outer helices of HEAT repeats 11 and 12, is stabilized in a closed conformation in exactly the same way as observed previously in the CRM1-RanBP1-RanGTP complex, the disassembly intermediate in the cytoplasm [4]. Close examination of the structure suggested that the internal loop of CRM1 (referred to as HEAT9 loop) is primarily responsible for maintaining the NES-binding cleft in a closed conformation and that the C-terminal tail of CRM1 stabilizes the autoinhibitory conformation of the HEAT9 loop and thereby reinforces autoinhibition.

Comparison of unliganded CRM1 with CRM1-NES-RanGTP complexes suggested that the binding of RanGTP induces movement of both the HEAT9 loop and the C-terminus of CRM1 and thereby enables the transition of the NES-binding cleft from the closed state to the open state.

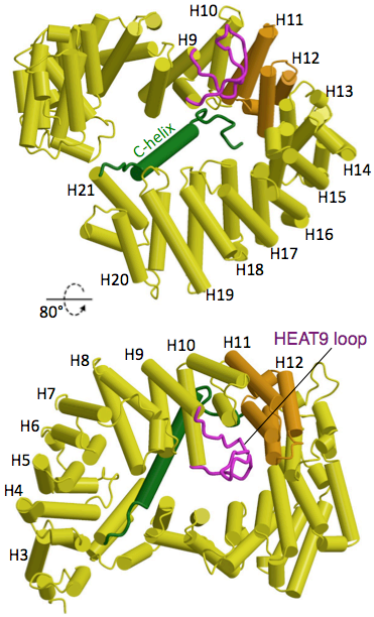

Fig. 1: Crystal structure of unliganded *S. cerevisiae* CRM1 (PDB code, 3VYC).

References

[1] T. Monecke *et al*., Science **324**, 1087 (2009).
[2] T. Guttler *et al*., Nat. Struct. Mol. Biol. **17**, 1367 (2010).
[3] N. Saito and Y. Matsuura, J. Mol. Biol. **425**, 350 (2013).
[4] M. Koyama and Y. Matsuura, EMBO J. **29**, 2002 (2010).

* matsuura.yoshiyuki@d.mbox.nagoya-u.ac.jp